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Labile and stable soil organic carbon and physical improvements using groundcovers in vineyards from central Spain

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Abstract

Mediterranean vineyards are usually managed with continuous tillage to maintain bare soils leading to low organic matter stocks and soil degradation processes. Vineyards are part of the Mediterranean culture and in order to make sustainable vineyards land use we propose the set up of two types of groundcovers. A field trial was performed to compare the effects of a seeded (*Brachypodium distachyon*) and spontaneous groundcovers, on a set of soil parameters, in comparison with the traditional tillage in four vineyards located in the center of Spain. Three years after the groundcovers establishment soil organic carbon stocks increased up to 1.62 and 3.18 Mg ha^{-1} for the seeded and the spontaneous groundcovers, respectively compared to conventional tillage. Both labile and stable fractions improved their soil organic carbon content with the use of groundcovers, particularly the labile fraction. Moreover, soil structure and functional soil properties improved through better aggregate stability, pore connectivity and infiltration rates. The higher root biomass input of the spontaneous groundcovers derived in higher soil organic carbon increases and soil quality improvement.

Keywords

Groundcovers; Soil organic carbon; aggregate stability; soil porosity; soil quality.

1. INTRODUCTION

Soil conservation is essential to supply goods, services and resources for the humankind (Keesstra et al., 2016). Agriculture causes several environmental impacts and particularly soil degradation processes (Bruun et al., 2015; Colazo and Buschiazzo, 2015). Among other factors, the severity and intensity of soil degradation is determined by the methods and techniques of managing soil systems (Lorenz and Lal, 2016; Kabiri et al., 2016; Zhang and Ni, 2017). In the context of agricultural land uses, Mediterranean vineyards undergo a series of soil degradation processes: soil erosion, organic matter loss, nutrient loss and, thus, a decreasing fertility trend. Soil erosion rates are especially high in Mediterranean vineyards reaching figures up to 100 Mg ha^{-1} (Prosdocimi et al., 2016; Rodrigo Comino et al., 2016; García-Ruiz, 2010). This can be explained by the scarcity of soil cover throughout the year (Lasanta and Sobrón, 1988). In addition, other circumstances are triggering high erosion rates: (1) vineyards are frequently planted on slopes (Arnaez et al., 2007), (2) rainfall intensities in this region can be high (Panagos et al., 2015), (3) soils used for growing vineyards have low organic matter content and therefore weak structure and (4) soil management usually consists in continuous tillage (Novara et al., 2011). Soil degradation results in particle detachment and organic matter and nutrient loss (Bienes et al., 2010), which makes soil aggregates progressively more sensitive to the impact of rain drops and causes soil crusts (Issa et al., 2006). The crust creates an impermeable layer that slows down water infiltration (Bu et al., 2014). When infiltration rate is slower than rain intensity, water runs off over the soil surface causing nutrient loss and soil particle detachment (Gómez et al., 2009; Ramos and Martínez-Casasnovas, 2004). Farmers usually remove these crusts by tilling as soon as possible after rains. Moreover each time that the farmer ploughs a fraction of soil organic matter is mineralized, soil aggregates became more easily destroyed and the crust formation will happen earlier during the forthcoming rainfalls. This way, Mediterranean vineyards experience continuous negative feedbacks caused by soil management practices.

The importance of soil degradation and erosion processes is usually underestimated. Not only because it causes on site and off site effects, but because decreases soil fertility, reducing crop yields (Bakker et al., 2004), and promoting land use changes (Bakker et al., 2005). At the beginning, soil damages mean costs for the farmers but in the end they turn into limitations and concerns for the socio-economic maintenance of the regions. Thus, it is critical to explore and encourage alternative soil managements to reduce soil degradation processes.

Soil organic matter (SOM) is one of the most important soil parameters because it enhances the global soil quality by improving physical, chemical and biological soil characteristics (Virto et al., 2012a; Ramos et al., 2010; Fernández-Ugalde et al., 2009). SOM is related to aggregate stability, water infiltration and soil fertility (Balesdent et al., 2000; Reeves, 1997). In this regard, climate change models agree that in the next decade the temperatures will raise in Mediterranean Basin, and this will increase organic matter mineralization rates (Pachauri et al., 2014). Taking into account that soils in Mediterranean vineyards have low organic matter content (Panagos et al., 2013), shifting conventional soil management practices to other more sustainable alternatives will counterbalance mineralization and will increase or maintain SOM.

When a fresh plant residue falls on the soil, it becomes a particulate organic matter (POM) for a while, before humification processes (Gregorich and Janzen, 1996) that will take place with different speed depending on environmental and residue characteristics to be transformed in SOM. Because of that, soil is enriched in SOM and nutrients and POM is considered as a good indicator of soil quality (Haynes, 2005). Besides, POM acts as a binding agent stabilizing macroaggregates (Six et al., 2002) which makes it an important variable to be considered to study soil management strategies in Mediterranean soils.

The soil structure concept reflects how soil particles are aggregated. As a physical soil characteristic, soil structure is a key factor to explain soil functioning (Bronick and Lal, 2005) and aggregate stability is commonly used as its indicator (Six et al., 2000a). SOM, microorganisms, clay, bivalent cations and ionic bridging are the main factors controlling soil aggregation. Soil aggregation is affected by the different organic matter inputs, amendments, soil management and tillage methods (Wei et al., 2006; García-Orenes et al., 2005). Consequently, different soil management strategies will affect aggregate stability differently.

Pore volume and pore size distribution are important factors in the soil-water relationships and soil aeration. Moreover, the biochemical processes take place in the soil pores (Jigău, 2012). Different soil management strategies can have an influence on the pore volume (Mulumba and Lal, 2008; Pituello et al., 2016) and distribution (Ruiz-Colmenero et al., 2013). Tillage breaks natural porosity and destroys the biological macropores made by the macro and mesofauna (Léonard et al., 2004) and by roots (Gao et al., 2017). Additionally, the roots of the vegetation cover create continuous pores enhancing infiltration (Glinski and Lipiec, 1990).

In this study, we use the term groundcovers (GC) for perennial crops instead of cover crops as is recommended by Gonzalez-Sanchez et al. (2015). The use of GC in Mediterranean vineyards is well known for reducing erosion rates (Prosdocimi et al., 2016; Duran Zuazo and Rodriguez Pleguezuelo, 2009), increasing soil organic carbon (SOC) (Virto et al., 2012b) or infiltration rates (Ruiz-Colmenero et al., 2013; Six et al., 2004). Temporal GC have proved to be a very effective tool for erosion control (Novara et al., 2011), but tillage, even if it is minimum, reduces the SOC threshold (García-Díaz et al., 2016). Consequently, the use of permanent GC is advisable in order to get significant and long term SOM increases.

In Europe, each country and region have the autonomy to develop their own agri-payments in the context of the Common Agricultural Policy to encourage sustainable soil management and, in this context, seeded and spontaneous GC use to have different considerations Some Mediterranean regions have developed successful policies to promote the use of GC in vineyards. In Sicily, where the most of the rainfall is recorded in autumn and winter, the farmers are paid for using winter cover crops. This management consists in seeded GC with leguminous species from October to April. In April, GC are mowed and tilled. This soil management practice is very effective controlling soil erosion and decreasing N loss (Novara et al., 2011; Novara et al., 2013). Similarly, in the Rioja Qualified Denomination of Origin's region, farmers receive different payments for using seeded or spontaneous GC (BOR, 2015). However, the majority of farmers in other regions, like the study area of this paper, even having problems of soil degradation, decline using GC in vineyards because of social stigmatization, culture (Marqués et al., 2015) and grape harvest reduction (Ruiz-Colmenero et al., 2011). Only the 3.1 % of the Madrid Region farmers use GC in vineyards (MAGRAMA, 2015a) which is one of the lowest percentages in Spain (MAGRAMA, 2015b). Moreover, in this region, the Common Agricultural Policy´s agri-payments do not consider the use of GC in vineyards in any case (BOCM, 2009).

Thus, we argue that more knowledge of the advantages and disadvantages of different GC and their effect on soil properties is needed to develop an efficient soil protection policy in European viticulture areas in different environmental conditions. Soil protection is also recognized as a long term strategy to sequester carbon and help to mitigate climate change (Poeplau and Don, 2015; Yagioka et al., 2015). Recently, the 4 per mille initiative has emerged to promote better soil management with the goal to increase global SOM stocks by 0.4% per year in order to compensate anthropogenic sources of global emissions of greenhouse gases (Minasny et al., 2017).

Frequently C sequestration rates are based on soil legacy data (Minasny et al., 2011; Akpa et al., 2016). Therefore a global effort on monitoring soil carbon "in situ" would be beneficial in obtaining the local soil conditions and SOC sequestration potential for particular environments as there is a critical limit to C sequestration which depends on soil texture and climatic conditions (Stockmann et al., 2015). In this regard, the SOC in 0.1 m depth in the vineyards of this study is going to be addressed as well.

With the main goal of studying the effect of two permanent GC (seeded and spontaneous) on soil properties and C sequestration in comparison with conventional tillage, we performed a trial including four different vineyards in the south of Madrid, with different soil characteristics which are representative of a large area of central Spain. We studied a set of soil parameters three years after the GC establishment: soil organic carbon (SOC) and the carbon of two fractions of the organic matter (particulate and associated to the mineral fraction); changes in other important soil characteristics were also monitored like aggregate stability; root stock, porosity, penetration resistance and infiltration rates. By including four different vineyards with different soils, slopes, orientations, vine varieties, prune systems, cultivation methods and microclimates we want to provide data on the effects of soil management strategy in different environmental conditions.

2. MATERIALS AND METHODS

2.1. Study area

The trial was performed in vineyards located in the Denomination of Origin “Wines of Madrid” (center of Spain). The annual rainfall is approximately 400 mm and the average temperature is 14.8 ºC. Four vineyards located on sloping terrain were selected in the municipalities of Campo Real, Belmonte de Tajo (two vineyards) and Navalcarnero. With the selection of these vineyards, we tried to represent the main soil types of the study area. The vineyards and the rows of the trials were also selected for having at least 9 consecutives inter-rows with the same soil and terrain characteristics. The Belmonte de Tajo vineyards are planted on a plateau on Miocene limestone parent material. Campo Real vineyard is located on ancient alluvial deposits with quartzite stones and clayey matrix. Navalcarnero vines are growing on soils over arkoses deposits resulted from the erosion processes of the Central Spanish Mountain Range. The altitude of the region is ranging from 586 to 783 meters above the sea level (Table 1), the slopes of vineyards of study are moderate (from 7 to 13.5 %).

Pits for soil classification and analyses were performed before the GC establishment in each vineyard. All soils were classified as Haploxeralfs (Soil Survey Staff, 2015). SOC contents and electric conductivity values were low (below 8.1 g kg^{-1} and less than 200 µS/cm). On the one hand, soils of Belmonte de Tajo and Campo Real vineyards are carbonated, which pH ranges from 8.5 to 8.6 and have active lime presence. On the other hand, Navalcarnero vineyard does not have carbonates nor active lime and its pH is 7.7. The cation exchange capacity is medium to low and textures were similar with the exception of the high sand percentage in Navalcarnero vineyard (Table 1).

Table 1. Soil Classification (Soil Survey Staff, 2015) and main soil characteristics of the vineyards. The trial had vineyards located in Belmonte de Tajo municipality (Bel-1 and Bel-2), Campo Real (CR) and Navalcarnero (Naval). The soil organic carbon (SOC), pH, electrical conductivity (EC), carbonates, active lime, texture class and cation exchange capacity (CEC) correspond to the Ap horizon. uS 1000mS 100 cS 10 dS cm 0.01 m

Site	**Soil Classification (USDA)**	**Altitude (m)**	**Slope (%)**	**SOC (g kg^{-1})**	**pH (1:2.5)**	**EC (μS cm^{-1})**	**Carbonates (%)**	**Active lime (%)**	**Clay (%)**	**Silt (%)**	**Sand (%)**	**CEC (cmol (+) Kg^{-1})**
Bel-1	Calcic Haploxeralf	754	7.2	8.1	8.5	162	27.8	8.7	24.0	31.5	44.5	14.9
Bel-2	Typic Haploxeralf	743	7.0	5.2	8.6	144	20.0	4.4	27.5	38.5	34.0	18.8
CR	Calcic Haploxeralf	783	13.4	7.2	8.6	160	16.1	6.2	31.5	18	50.5	12.2
Naval	Typic Haploxeralf	586	13.5	7.0	7.7	145	0.1	<0.1	24.5	8.5	67.0	14.0

2.2. Experimental design

The trial started at the end of 2012 with the selection of the four studied vineyards. The treatments were: i) conventional tillage (T) consisted in 2-3 tillage operations per year with chisel (15 cm depth) following the typical soil management practice of this area; ii) *Brachypodium distachyon* groundcover (hereafter CB), seeded in the central 2 m of the inter-rows in December 2012, being the seeding rate 20 kg ha^{-1}; and, iii) spontaneous vegetation cover (hereafter CS). Both vegetation cover treatments (CB and CS) were mowed every spring once or twice a year, depending on the rainfall regime, at the height of 10 cm. The resulted straw residue or litter was left on the soil surface. This cut height allowed the CB treatment to auto-seed. The 3 treatments were performed in all 4 sites with 3 repetitions according to (García-Díaz et al., 2017).

2.3. Soil sampling and laboratory methods

Three years after the beginning of the trial, in the summer of 2015, 9 soil samples per treatment were collected from the 4 vineyards at 0 to 5 cm and 5 to 10 cm depth. Soil samples were air-dried and sieved to obtain various sized fractions to carry out soil tests as described below. Texture was determined with Robinson pipette method.

Soil organic carbon can be found in different forms and potentials to be stable or recycled, thus the need to identify stable and labile fractions. The organic matter content associated to soil mineral fraction is considered as the stable fraction of organic carbon. This mineral associated organic fraction was separated from the total organic matter by dispersing 10 g of the 2 mm sieved fraction in 30 mL of sodium hexamethaphosfate (5 g L^{-1}) and further shaking for 15 h. Then, the dispersed soils were wet sieved at 50 µm mesh size and the different fractions were oven-dried at 50ºC (Cambardella and Elliott, 1992). SOC and mineral-associated organic carbon (MOC) were determined by (Walkley and Black, 1934) method. Soil organic carbon of the particulate organic matter (POC) was obtained as the difference between SOC and MOC.

SOC, MOC and POC stocks were obtained based in Equation 1.

$$\text{C Stock (Mg ha}^{-1}\text{)} = [\text{SOC, MOC or POC}] \times \text{BD} \times \text{D} \times 10 \qquad \text{(Eq. 1)}$$

where: SOC is soil organic carbon (fraction < 2 mm) (g kg^{-1}); MOC is the mineral-associated organic carbon (g kg^{-1}); POC is particulate organic carbon (g kg^{-1}); BD is the bulk density (Mg m^{-3}); and D is the sampling depth (m).

Plant roots content in percentage was determined in undisturbed soil samples obtained by drill sampling (Ø=2.18 cm) at 0-5 and 5-10 cm depth taking 9 samples per treatment. Samples were air dried, weighed and wet sieved at 0.2 mm mesh size. Then, water and roots were vacuum filtered with a Büchner funnel and Kitasato flask over a tared paper filter. The obtained filters with roots were oven-dried at 105ºC for 24 h and weighed. The root stock was calculated similarly to Eq. 1 but C stock and content were substituted by the root stock and content.

There are several methodologies to assess aggregate stability (Hernanz et al., 2002; Imeson and Vis, 1984) and a number of researches have addressed the increase in aggregate stability with organic amendments (Hontoria et al., 2016; Peng et al., 2016; Rahman et al., 2017) or the use of cover crops (Ruiz-Colmenero et al., 2011; Salomé et al., 2016). Most of them include only one aggregate stability test. Knowing that the results can be different by using different methodologies, we propose including two widely used aggregate stability tests to assess their efficiency to show the effects of different soil management strategies on aggregate stability and, thus, soil structure.

The first method was performed on macroaggregates by counting the number of drops (CND) needed to break aggregates. An aliquot of soil samples was dry sieved to obtain macro-aggregates with diameter size between 4 to 4.75 mm (Boix-Fayos et al., 2001) to perform the CND test. Thirty macro-aggregates of each soil sample (9 samples per treatment) were used to perform resistance to drop impact test (Imeson and Vis, 1984) by this CND test. The second method was the water stable microaggregates (WSA) test, that was expressed as the percentage of the <2 mm aggregates resistant to wet sieving (Kemper and Rosenau, 1986). Aggregate samples were submerged and emerged over a 0.25 mm sieve at 30 oscillations per minute during 3 minutes. These calculations were corrected for sand content. Three subsamples of 5 g were evaluated from each of the soil samples (9 samples per treatment).

2.4. Soil cores: macro, meso, microporosity, available water holding capacity and bulk density

To determine the macro, meso and microporosity, total porosity, available water holding capacity (WHC) and bulk density, 9 undisturbed soil cores per treatment were taken in the 4 vineyards. The cores have 5 cm of diameter and are 5.1 cm high (100 cm^3). These cores were taken with the objective of sampling the first 5 cm of depth, however, to ensure that 100 cm^3 were exactly sampled, the first 2 to 3 cm of the surface soil were removed to avoid irregularities mainly caused by the vegetation. Consequently, the final sampled depth was between 2-3 and 7-8 cm deep.

The cores were water saturated by capillarity in a sandbox. Then, increasing suctions from pF 0 to pF 4.2 were applied. In the sandbox pF ranged from 0 to 2, and in the pressure plates system (Richards, 1941; Richards, 1965) pF ranged from 2.54 to 4.2. As the last step, samples were completely oven dried (24 h at 105ºC) to calculate the bulk density. We define the different pore size groups following the classification suggested by Taboada et al., (2004) which separates macropores (those pores with diameter > 60 µm; pF from 0 to 1.8), mesopores (those pores between 60 and 10 µm; pF from 1.8 to 2.54) and micropores (those pores ≤ 10 µm; pF>2.54). WHC was calculated as the difference between the moisture held in soil microporosity and that of the permanent wilting point (pF = 4.2). Not available water (NAW) was calculated as water content below the permanent wilting point. pF data were also used to determine water retention curves. We used RETC software (van Genuchten et al., 1991) to calculate the parameters of the empirical equation Eq. 2 modified by van Genuchten and Nielsen (1985).

$$\theta_h=\theta_r+(\theta_s-\theta_r)[1+(\alpha h)^n]^{-m} \qquad \text{(Eq. 2)}$$

Where θ_h is the water content at suction pressure h (m^3 water m^{-3} soil); θ_r is the residual water content (m^3 water m^{-3} soil); θ_s is the saturated water content (m^3 water m^{-3} soil); α is the inverse of the air entry suction (m^{-3}); n is a dimensionless value related with the pore size distribution; m is 1-(1/n); and h is the suction pressure.

Intrapedal porosity was assessed using the petroleum method (Mursec, 2011). Macroggregates (4-4.75 mm diameter) were weighed, submerged in petroleum oil during 24 h to allow pores to infill. The mass difference was used to calculate the intrapedal porosity.

2.5. Field tests and determinations

Infiltration rates were obtained with a simple-ring infiltrometer of 12.5 cm of diameter (USDA, 1998) by recording the time necessary to infiltrate 25 mm of water and repeating it for 10 times. Nine infiltration tests were performed on each treatment of each vineyard (3 treatments x 4 vineyards x 9 infiltration test).

Penetration resistance was assessed using an Eijkelkmamp® penetrometer. It was carried out 27 times per treatment for the four vineyards at depths of 2.5, 5, 10, 15, 20, 25, 30, 35, 40 and 45 cm.

The vegetation cover percentage was determined in three random areas along each row of each treatment (3 spots x 3 rows x 3 treatments x 4 vineyards) at the beginning of the summer using 25 x 25 cm^2 quadrats. At each spot, the average of 6 observations from 6 trained observers was used to determine the percentage of vegetation cover. This methodology results in similar values than those obtained employing digital image analysis (García-Estríngana et al., 2005).

2.6. Data analysis

Before data analyses, variables were checked and transformed to ensure normal distribution of data if needed. Homocedasticity was also checked. The analyses were developed in different stages using Statistica 10 software (StatSoft Inc., 2011). First, we conducted a variance analysis (Factorial ANOVA). Factors were the soil treatment (CB, CS and T), site (the four studied vineyards) and depth (0-5 cm and 5-10 cm). The dependent variables were SOC, MOC, POC, CND, WSA, root stock, BD, intrapedal porosity and vegetation cover. After that, the variables were analyzed by separating depths, except BD and intrapedal porosity as they have been studied for the same depth. Considering that penetration resistance was measured for many depths we performed simple ANOVA at each depth with soil treatment as the only factor. Further, macro, meso, microporosity, WHC, NAW and infiltrations were analyzed with simple ANOVA with soil treatment as a factor. Canonical analyses were used to assess the relationships between soil treatments (CB, CS and T) and dependent variables. Soil treatments were previously codified as dummy variables to include them in canonical analysis.

3. RESULTS

3.1. Soil organic carbon and fractions

The factorial ANOVA showed that SOC, MOC and POC were strongly affected by soil treatment and site (Table 2). Depth had influence in SOC and MOC but not in POC. The interaction [Treat x Site] was strongly significant to SOC and lower to MOC. [Treat x Depth] and [Site x Depth] had overall low signification and only POC was highly influenced by [Site x Depth]. Again, POC was the only organic carbon fraction with a big influence of the interaction [Treat x Site x Depth].

Table 2. Results from the analysis of variance for soil organic carbon (SOC), mineral-associated organic carbon (MOC), soil carbon of the particulate organic matter (POC) fraction. The treatments were CB (*Brachypodium distachyon*), CS (Spontaneous Vegetation) and T (Conventional Tillage). Sites were vineyards in Belmonte de Tajo (two vineyards), Campo Real and Navalcarnero. Depth were 0-5 and 5-10 cm. Terms with p value >0.1 were not reported (NR). N=36.

	Treat	**Site**	**Depth**	**Treat* Site**	**Treat* Depth**	**Site* Depth**	**Treat* Site*Depth**
SOC	<0.001	<0.001	<0.001	0.001	0.091	0.014	0.051
MOC	<0.001	<0.001	<0.001	0.011	NR	NR	0.040
POC	0.004	<0.001	0.083	NR	0.047	0.003	0.001

For both studied depths the differences between treatments were the same for SOC, MOC and POC: CS showed significantly higher carbon values than T, while CB did not differ from CS and T (Fig. 1). MOC stocks for the different treatments were 8.85, 9.72 and 8.03 Mg ha^{-1} for CB, CS and T, respectively, which means a relative increase of 10

% for CB and 21 % for CS compared to T. POC contents were 4.06, 4.75 and 3.25 Mg ha^{-1} for CB, CS and T, respectively. Three years after introducing groundcovers CB increased SOC in 1.62 Mg ha^{-1} year^{-1} and CS did it in 3.18 Mg ha^{-1} year^{-1} regarding T.

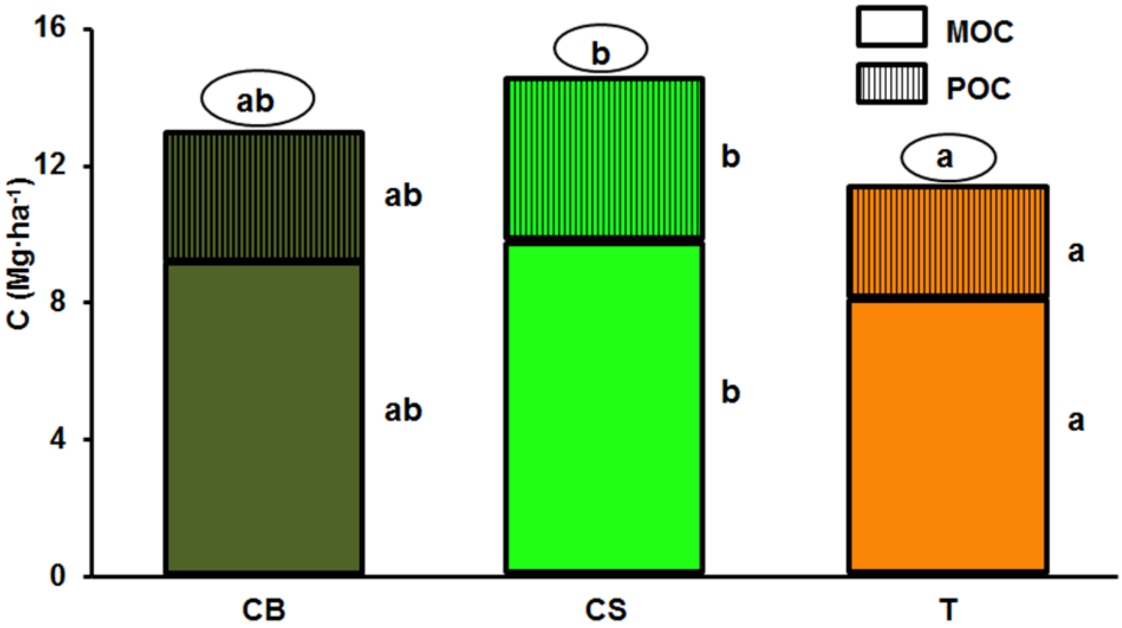

Figure 1. Mean of soil organic carbon (SOC), mineral-associated organic carbon (MOC) and soil organic carbon of the particulate organic matter fraction (POC) for the different treatments at 0-10 cm depth. CB (*Brachypodium distachyon*), CS (Spontaneous Vegetation) and T (Conventional Tillage). Different letters mean significant differences between treatments at $p<0.05$. Surrounded letters refer just to SOC statistical differences. N=36.

3.2. Soil parameters and its relations to management strategies

After three years of GC establishment all variables were significantly affected by the soil treatments at $p<0.05$ and strongly affected by the site at $p<0.001$ (Table 3). Soil depth had influence in WSA and root stocks. The interaction [Treat x Site] was strongly significant on CND but had lower signification on WSA and root stocks. [Site x Depth] was significant for the roots while [Treat x Site x Depth] did not influence any variable. CS increased strongly the CND doubling the average of T while CB scored similar values than T. However, both GC treatments increased the WSA significantly. The root stocks was increased in CS by more than 3 Mg ha^{-1} while CB also showed higher values than T but not statistically significant. CB showed the higher values of bulk density and CS did not differ from T. CB increased significantly intrapedal porosity in soil while CS did not.

Table 3. Mean and standard deviation of soil parameters for the different treatments and results from the analysis of variance for counting number drops aggregate stability (CND) test, water stable aggregates (WSA) test, root stock and bulk density. The treatments were CB (*Brachypodium distachyon*), CS (Spontaneous Vegetation) and T (Conventional Tillage). Sites were vineyards in Belmonte de Tajo, Campo Real and Navalcarnero. Depths were 0-5 and 5-10 cm. Different letters mean significant differences between treatments for the 0-10 cm depth at p<0.05. Terms with p value >0.1 were not reported (NR).

	Treatment			**Effect**						
	CB	**CS**	**T**	**Treat**	**Site**	**Depth**	**Treat* Site**	**Treat* Depth**	**Site* Depth**	**Treat* Site*Depth**
CND	10.41 ± 0.76 a	20.14 ± 2.67 b	9.14 ± 0.85 a	<0.001	<0.001	NR	0.001	NR	NR	NR
WSA (%)	41.63 ± 1.56 b	43.01 ± 1.81 b	37.11 ± 1.46 a	<0.001	<0.001	<0.001	0.023	NR	NR	NR
Root Stock (Mg ha^{-1})	7.39 ± 8.04 ab	9.55 ± 11.58 b	6.04 ± 6.02 a	<0.001	<0.001	<0.001	0.019	NR	<0.001	0.036
Bulk density (Mg m^{-3})	1.42 ± 0.02 b	1.37 ± 0.03 ab	1.34 ± 0.02 a	0.049	<0.001	-	NR	-	-	-
Intrapedal porosity (%)	36.61 ± 0.76 b	34.61 ± 0.90 a	34.14 ± 0.84 a	0.011	<0.001	-	0.096	-	-	-

3.3. Depth and soil management

Both GC showed higher SOC values at 0-5 cm depth than at 5-10 cm (Fig. 2) meaning that a stratification of SOC is taking place. Regarding MOC only CS showed statistically different values between depths. POC values did not differ between depths. SOC, MOC and POC showed the same trend of differences between treatments at the studied depths: CS had significantly higher values than T with CB showing no differences with any treatment at 0-5 cm depth. The aggregate stability tests did not show differences between depths for any treatment with the exception of WSA in T. No statistical differences were found for WSA when separating between depths. The CND test had significantly higher values for CS than CB and T in the two selected depths. At 5-10 cm depth, no statistical differences between treatments were recorded for any variable. Regarding root content, only CB showed statistically significant higher values at 0-5 cm depth than at 5-10 cm depth. Again, at 0-5 cm depth only CS differs from T.

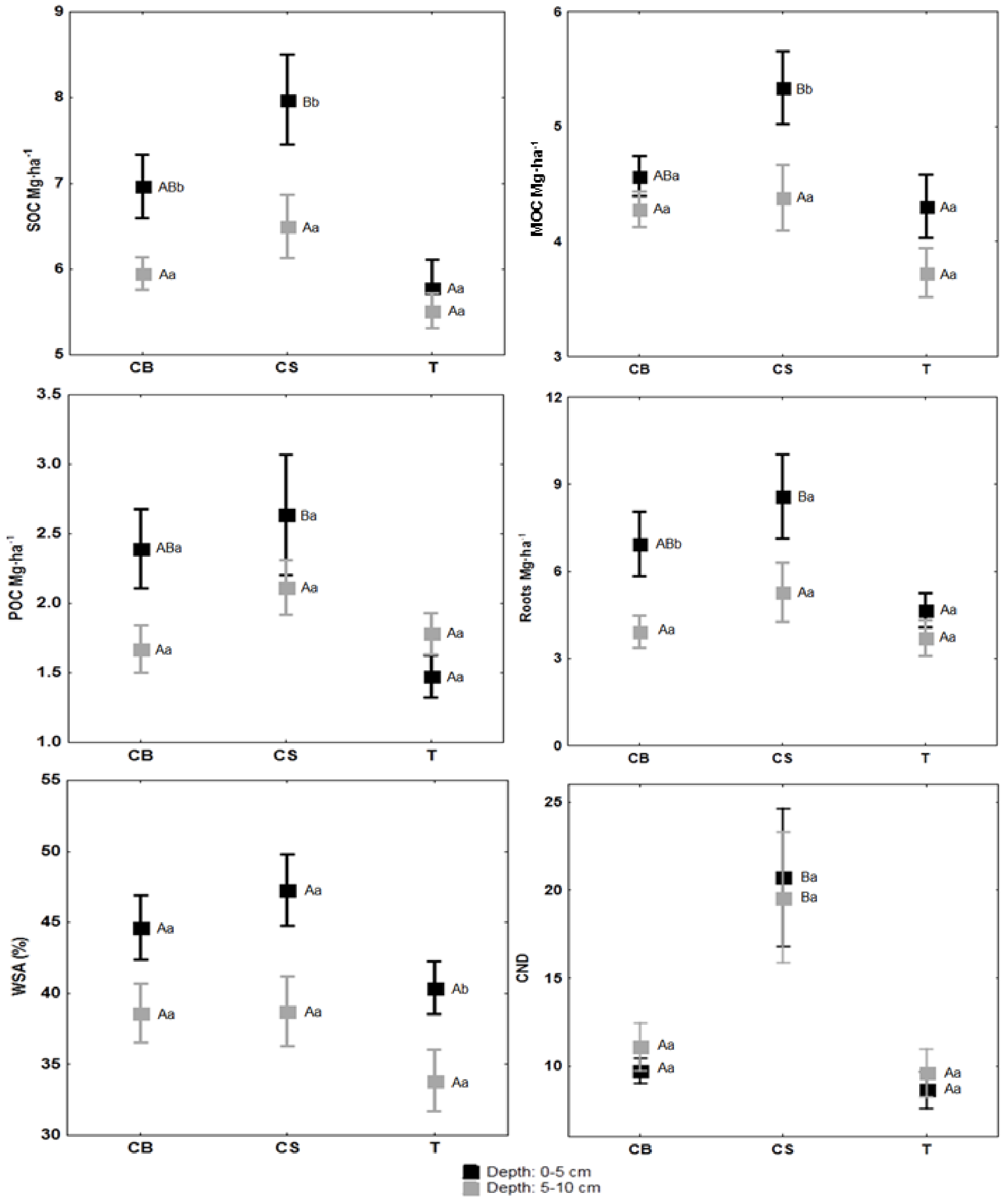

Figure 2. Mean and standard error of soil organic carbon (SOC), mineral-associated organic carbon (MOC), soil organic carbon of the particulate organic matter fraction (POC), counting number drops aggregate stability test (CND), water stable aggregates test (WSA) and root stock for the different treatments and depths after three agronomy seasons. CB (*Brachypodium distachyon*), CS (Spontaneous Vegetation) and T (Conventional Tillage). Different uppercase letters mean differences between treatments at the same depth at $p<0.05$. Different lowercase letters mean differences between depths at the same treatment at $p<0.05$. N=36.

3.4. Penetration resistance

Results were separated in two sections (Fig. 3): the first section from 0 to 20 cm depth, with significant differences in penetration resistance, and the second section from more than 25 to 45 cm depth where no statistical differences were observed. Section 1 showed that T scored the lower penetration resistance than both GC treatments. From 0 to 15 cm depth, the penetration resistance of groundcovers doubled the values of the tilled soils. At the depth of 20 cm T and CS had no differences. In section 2, the values were erratic along different depths and had very high variability which resulted in a lack of statistical differences.

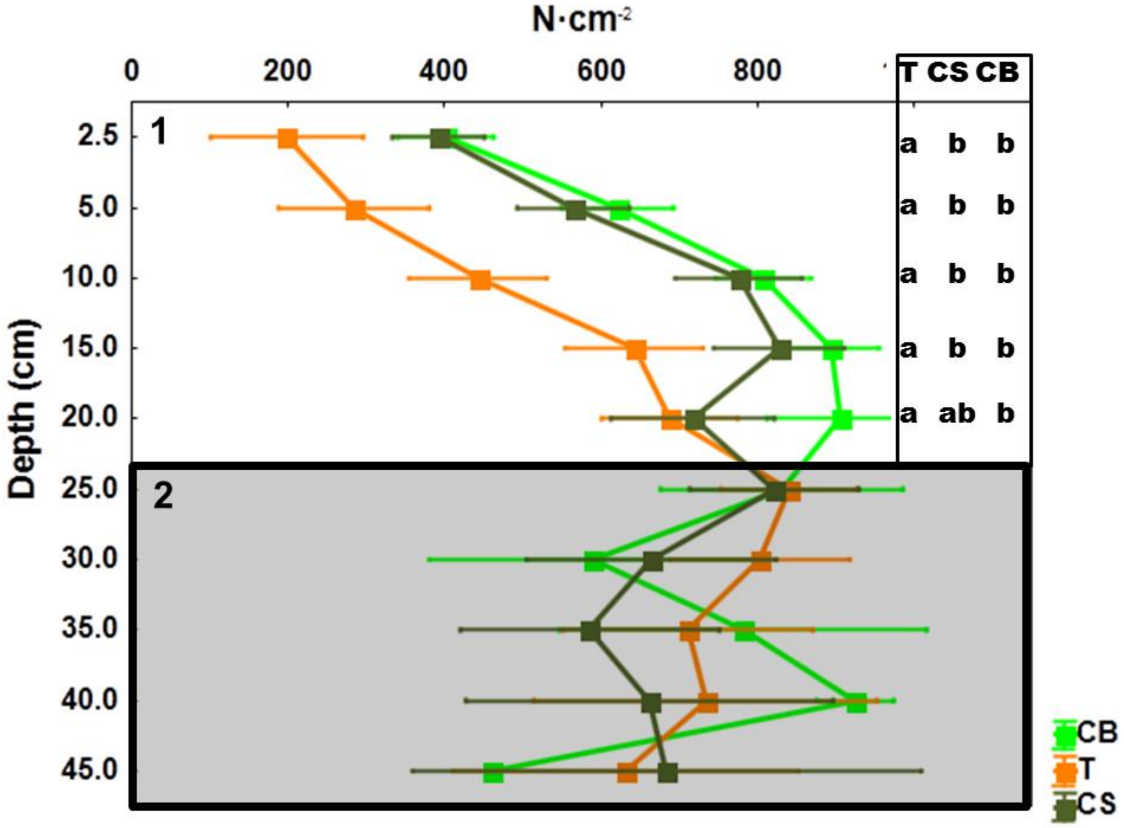

Figure 3. Mean and standard error of penetration resistance for the different treatments after three agronomy seasons at different depths. CB (Brachypodium distachyon), CS (Spontaneous Vegetation) and T (Conventional Tillage). Different letters mean significant differences between treatments at $p<0.05$. Section 1: with statistically significant differences. Section 2: without statistically significant differences.

3.5. Porosity and infiltration

Total porosity of sampled cores after three years of soil treatments did not change significantly (Fig. 4) between treatments. CS scored the highest average with 47.3 % followed by CB (46.2%) and T (45.8%). CS and T had significantly higher macroporosity than CB. However, CB had higher micro-porosity than CS and T, which was reflected in a higher WHC. The analysis of Fig. 4 shows that three years of GC development have been enough to reorganize the soil pore structure, although total porosity does not change.

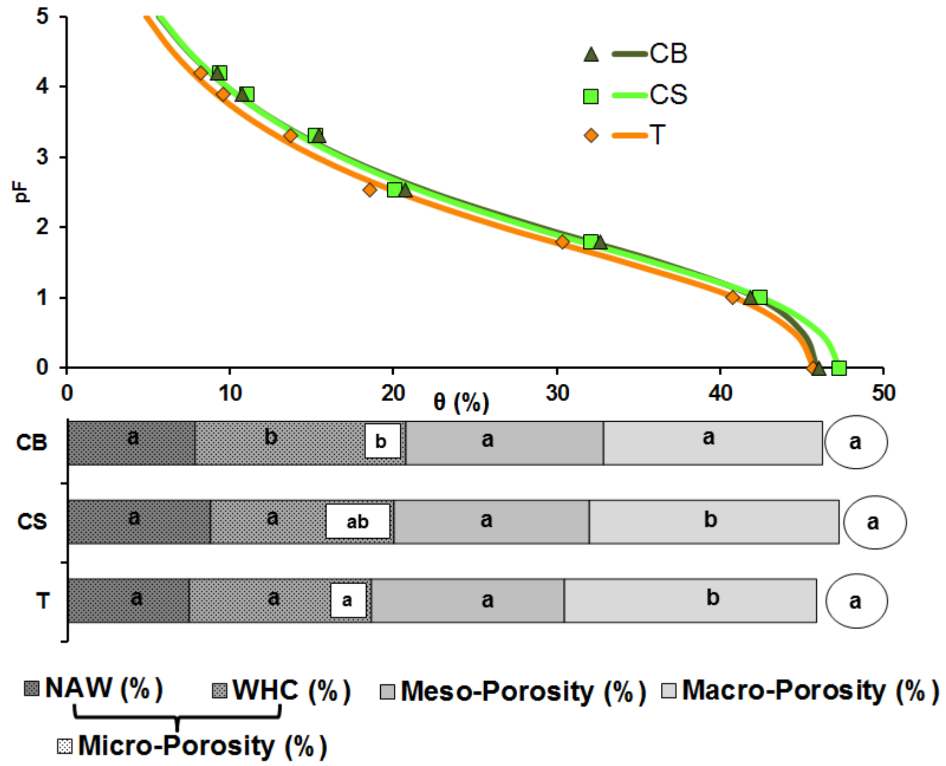

Figure 4. Upper part: water retention curve for the three treatments three years after the start of the trial. θ= volumetric water content. Lower part: mean values of volumetric water content for the three treatments. Total porosity is divided in macroporosity, mesoporosity and microporosity. Microporosity was divided in available water holding capacity (WHC) and not available water (NAW). CB (*Brachypodium distachyon*), CS (spontaneous vegetation) and T (conventional tillage). Different letters mean significant differences between treatments at $p<0.05$. Letters surrounded by a square refer to microporosity differences. Letters surrounded by a circle refer to total porosity differences. N=36.

With regard to infiltration rates, during the first minutes of the experiments all the treatments showed similar rates starting with 80 cm h^{-1}. However, CS and CB maintained higher values for 0.15 hours after the beginning (Fig. 5). The steady state values of CS and CB were 28.1 and 23.3 cm h^{-1}, respectively, which were statistically higher than T, with 13.1 cm h^{-1}. Thus, in three years, groundcovers improved infiltration rates by increasing them ca. 2 times.

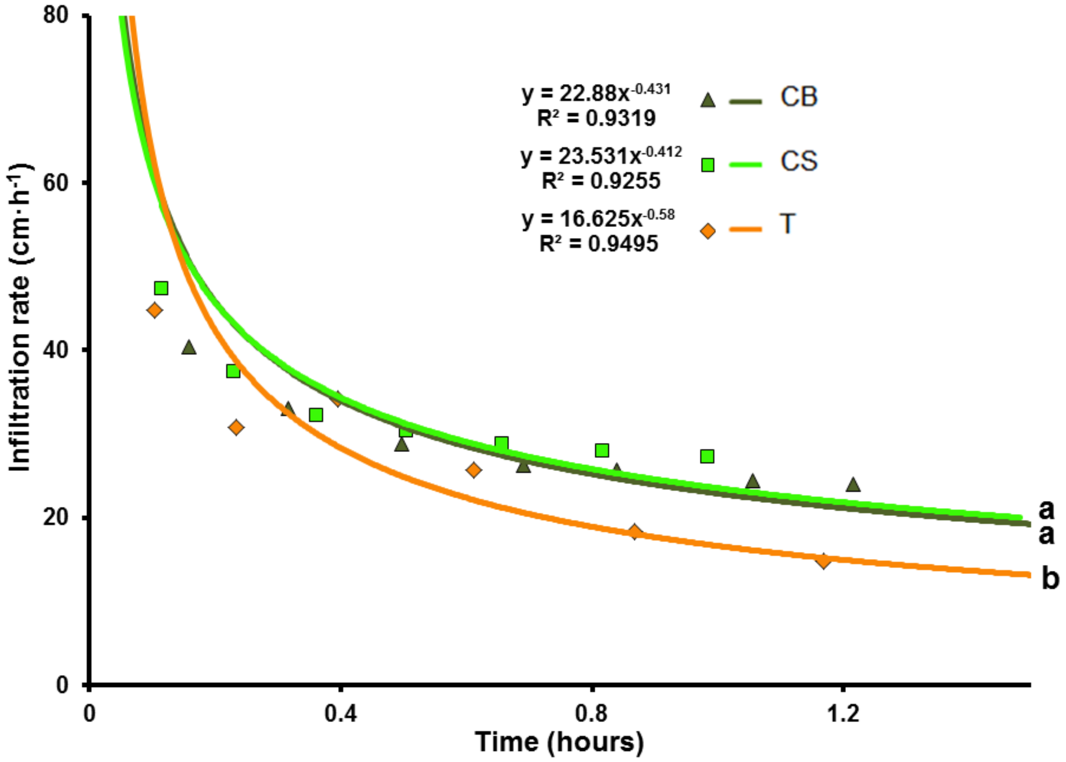

Figure 5. Infiltration curves for the three treatments three years after the start of the trial fitted to potential equations. CB (*Brachypodium distachyon*), CS (spontaneous vegetation) and T (conventional tillage). Different letters mean significant differences between steady states of the treatments at $p<0.05$. N=36.

3.6. Relationships between parameters

The relationship between SOC and aggregate stability is especially important in Mediterranean vineyards as explained in the Introduction section and deserves further analysis. A first exploration of the variables correlation matrix (supplementary material) showed that the aggregate stability tests scored the highest correlation indexes with SOC instead of POC and MOC. Thus, correlation analyses were performed with SOC and aggregate stability tests.

CND and WSA showed a positive and significant correlation with SOC ($p<0.05$) (Fig. 6) with and adjusted R^2 of 19.5 and 38.6 %, respectively. These figures indicated that with low SOC values, a small increase strongly improves the aggregate stability. On the contrary, with higher SOC, a bigger increase is needed to promote aggregate stability. This is remarkable in the correlation established between WSA and SOC (Fig. 6b), in which an asymptotic equation could be observed. On the contrary CND showed an almost lineal relationship with SOC.

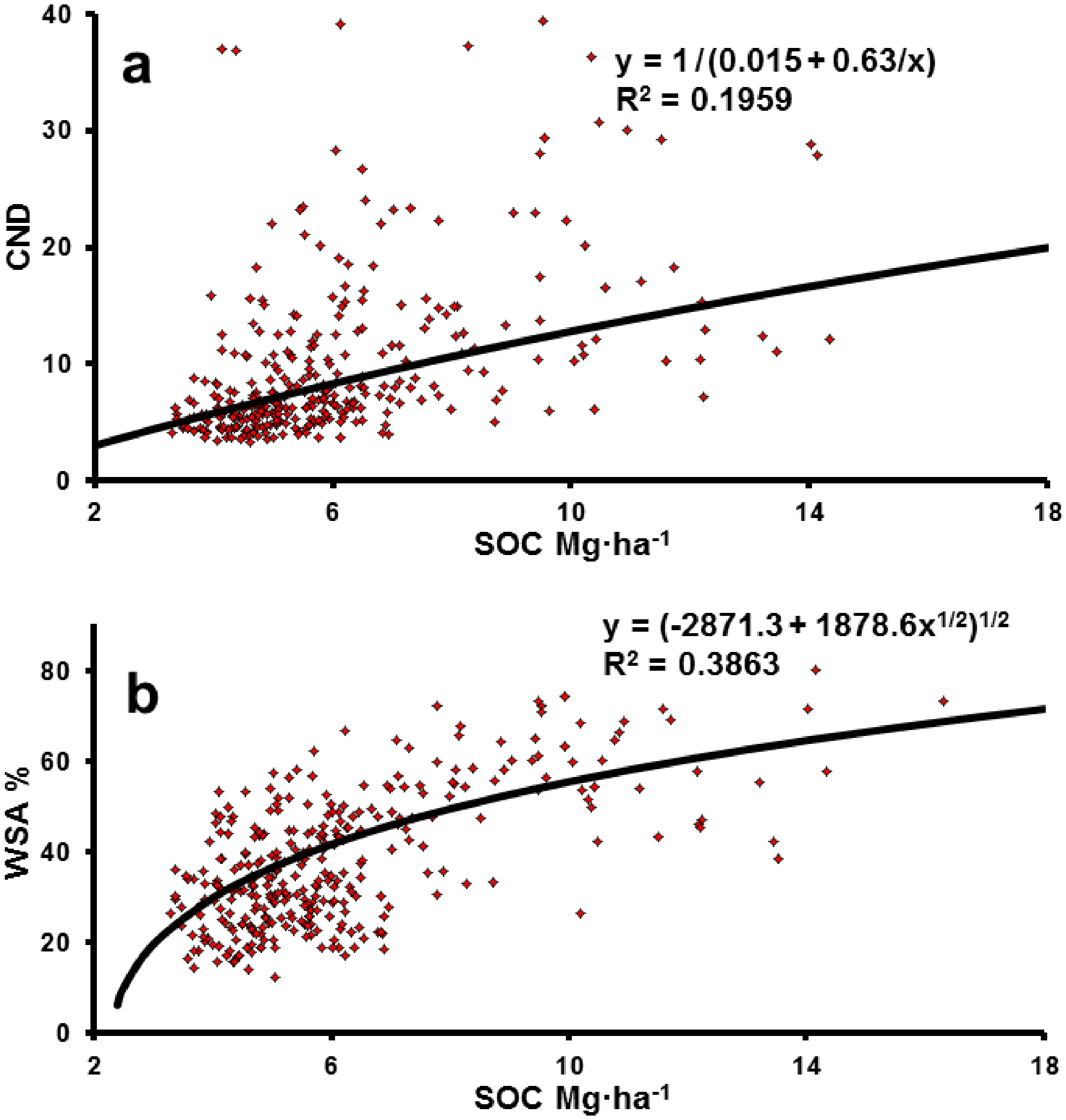

Figure 6. Relationships between soil organic carbon (SOC) at 0-10 cm depth and (a) counting number drops (CND); and (b) water stable aggregates (WSA) with the best fitting equations, which are statistically significant at $p<0.05$.

Due to the multiple correlations found between variables, a multivariate canonical analysis was performed with selected variables that minimize co-variation, site-dependence relationship and redundancy. The model included: the treatments CB, CS and T, and the variables: POC, MOC, CND, WSA, root stocks, PR, Infiltration, intrapedal porosity, total porosity, macroporosity, mesoporosity, WHC, bulk density and vegetation cover. The canonical analysis extracted 61 and 38 % (roots 1 and 2, respectively) of the variability of the independent variables and 14 and 10 % (roots 1 and 2, respectively) of the dependent variables. This relatively low explained variability is due to the large number of variables that were included in the canonical analysis. To facilitate interpretations, two graphs were made with the ordination resulted from canonical analysis (Fig. 7a and b). The first one was focused on organic matter and aggregate stability and the second one on variables related to the water in the soil. Roots and vegetation cover were included in both graphs because they had big influence in the two sets of variables.

Vegetation cover scored positive in Root 1 as well as both GC treatments. Fig. 7a showed similar scores for the organic matter fractions, aggregate stability and roots in Root 1, which explains the most of the variability and is linked to the GC. All dependent variables but particularly CND scored positives values in Root 2, which positioned them closer to CS than to CB. Figure 7b showed that soil treatments had very low influence on mesoporosity. The macroporosity was negatively correlated with CB. Total porosity was slightly affected by soil treatments but scored more similar to groundcovers than to T. WHC and intrapedal porosity were highly related and scored close to BD. These variables scored positive in Root 1 as well as groundcovers but negative in Root 2. Vegetation cover was related to penetration resistance, infiltration rates and roots content in the soil. Higher vegetation cover meant higher penetration resistance or compaction. However, contrary to expectations, this did not lead to infiltration problems, instead, the infiltration rates were higher as the vegetation cover and penetration resistance values went higher. Taking into account that groundcover did not increase the pore volume (Figs 4 and 7b) we can conclude that pore connection is more important than pore volume to explain infiltration rates and that soil compaction is not directly related to water infiltration in this study. GC, and especially CS, increase the vegetation cover percentage, which increases the soil roots content. This higher root biomass input increases the organic carbon (both fractions) and improves the aggregate stability.

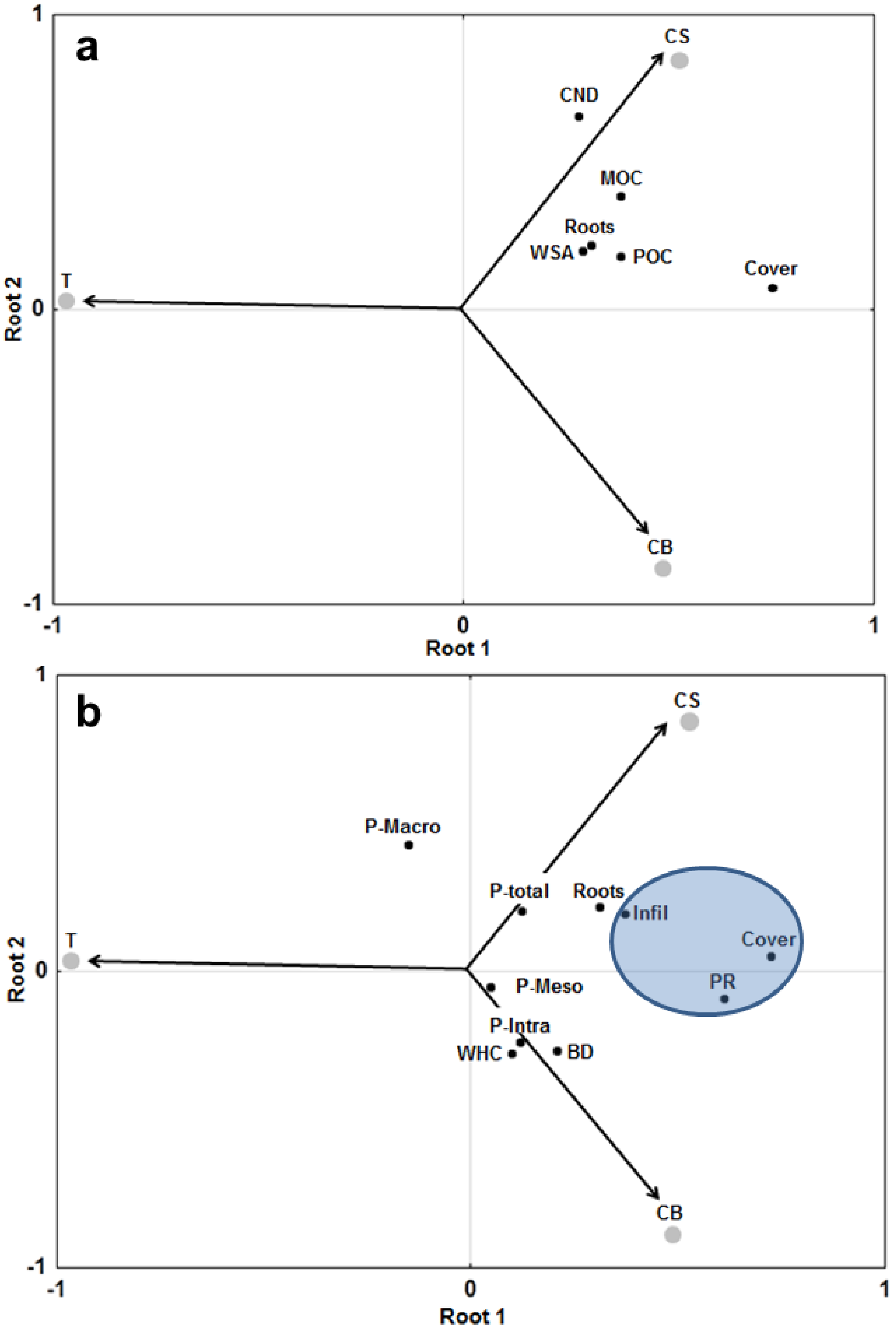

Figure 7. Spatial ordination resulted from canonical analysis. *Brachypodium distachyon* groundcover (CB), spontaneous vegetation groundcover (CS), conventional tillage (T), soil organic carbon of the particulate organic matter fraction (POC), mineral-associated organic carbon (MOC), counting number drops macroaggregate stability test (CND), water stable microaggregates test (WSA), root stocks (Roots), penetration resistance (PR), infiltration (Infil), intrapedal porosity (P-Intra), total porosity (P-Total),

macroporosity (P-Macro), mesoporosity (P-Meso), available water holding capacity (WHC), bulk density (BD) and vegetation cover (Cover).

4. DISCUSSION

The site of vineyards had the strongest influence on the studied variables as expected. However, this study aimed at including soil and location variability in order to contrast them with the different soil management practices to prove whether a soil management strategy can improve soil quality, no matter the type of soil. Three years after the beginning of the GC establishment the studied soil properties experienced some changes. Soils with CS had significantly more SOC, MOC and POC than T, with CB scoring intermediate values. The most important increase corresponds to 0 to 5 cm soil depth. At 5 to 10 cm depth there were just tendencies but not statistically significant changes. Usually, at deeper layers there were no significant differences between conventional tillage and other sustainable soil management practices (Piccoli et al., 2016; Powlson et al., 2011).

Our results showed that CS increased soil carbon in both stable and labile fractions. The importance of this result is that GC promote atmospheric CO_2 sequestration in the soil via increasing MOC stocks (Stockmann et al., 2013). At the same time soil quality and fertility are improved (Gregorich et al., 1997) and erodibility is reduced (Parras-Alcántara et al., 2016) via increasing POC stocks.

CS and CB had 1.06 and 0.54 Mg SOC ha^{-1} $year^{-1}$ sequestration rates, respectively. These results are consistent with those published by Vicente-Vicente et al., (2016) who performed a meta-analysis including 16 publications on vineyards and found an average of 0.78 Mg SOC ha^{-1} $year^{-1}$ while for olive orchards the rates were 1.1 Mg SOC ha^{-1} $year^{-1}$. Peregrina et al., (2010 and 2014) observed SOC sequestration rates of 0.47 and 1.19, and 1.34, 1.52, Mg SOC ha^{-1} $year^{-1}$ in different trials for different types of permanent GC in vineyards. More importantly, these stocks demonstrate that the 4 per mille objective can be accomplished. The relatively low values found for CB could be attributed to the lower biomass inputs that were scored in the case of the root stocks. Moreover, it is difficult to compare SOC sequestration rates because sometimes the soil management strategy is called cover crops or groundcovers but these strategies include an annual tillage for seeding operations or to limit water competition obviating that tillage is one of the major driving forces of carbon mineralization (Kabiri et al., 2016). Additionally, there is not a standard of soil sampling depth to calculate SOC stocks or sequestration rates. CB increased POC stocks in 0.8 Mg ha^{-1} and CS did in 1.5 Mg ha^{-1} in relation to T. The increase in POC and its relationship with higher soil quality has been widely addressed in the literature (Duval et al., 2013). Moreover, though most of the SOC belongs to the stable fraction (MOC), GC increased relatively more POC stocks than MOC.

The aggregate stability tests showed different results. Regarding the CND, only CS averaged significantly higher than T while both GC had higher values of WSA. T showed the lower values of any soil treatment in both test. As tillage destroys the natural structure of soil, it produces more unstable macroaggregates because of lower SOC contents (Six et al., 2000b). Therefore, any increase in aggregate stability is related to higher SOC contents (Blavet et al., 2009) and especially to the POC content (Imaz et al., 2010). This labile organic matter content is more sensitive to land management changes than SOC (Bayer et al., 2004). In this study, the WSA test was similarly influenced by MOC and POC in the canonical analyses, while the CND was closer to MOC. Higher POC is related to a continuous replacement of fresh organic matter that can be incorporated into the soil (Sá and Lal, 2009). Besides, POC acts as a binding agent to stabilize macroaggregates (Denef et al., 2004; Six et al., 2002). This could suggest that stable organic carbon increases the resistance of aggregates to raindrop impacts and thus, decreases soil erodibility.

Both aggregate stability tests were positively correlated with SOC, which was observed by several researchers (Bronick and Lal, 2005; Six et al., 2004), but the correlations were different. CND followed a nearly linear distribution while the one of WSA was asymptotic. This could be explained by the fact that the WSA had higher sensitivity to a SOC increase when the initial SOC content was low. In fact, Minasny et al., (2017) have established in more than 20 countries that there is a tendency of a higher C sequestration potential on croplands with low initial SOC stock. In this study with soils having low SOC, WSA is more suitable to assess soil quality changes, hence its use is recommended for the assessment of soil status of Mediterranean vineyards.

The cessation of tillage and mowing operations are often related to soil compaction (Lagacherie et al., 2006). Accordingly, our data showed that GC had significantly higher penetration resistance than T in the tilling depth. Nevertheless, this compaction had no consequences on the soil-water and soil-air relationships. As soil water retention curves and multivariate analysis showed, GC did not have a strong impact on soil porosity. Total porosity was statistically the same for all treatments, in fact CS tended to be even higher. GC developed a new pore distribution with similar pore volume but a higher connectivity. BD was higher in the GC than in T showing higher soil compaction that was also proved by higher values of penetration resistance field tests. Nevertheless, even with higher soil compaction and similar porosity, GC doubled the infiltration rates of T. Vegetation cover, penetration resistance and infiltration scored similarly in the canonical analysis, this indicates that soil compaction itself is no enough to explain the water movement in the soil. Our results agree with those from Pires et al., (2017) and Gao et al., (2017) who observed small differences in macroporosity, but better pore connectivity under no tillage treatment. Thus, despite soil compaction, GC improves the water movement without threatening soil aeration.

In this regard, CB had behaved slightly different than CS: macroporosity was lower and microporosity and WHC were higher in CB. The intrapedal porosity was also higher in CB which explained the higher proportion of small pores and the enhancement of WHC. It is well known that different patterns in soil roots result in different soil structure and pore size distribution (Krebs et al., 1993) and that traditionally tilled plots usually show less intrapedal porosity (Aguilar et al., 1990).

The most of the studied variables were not affected by soil treatments at 5 to 10 cm depth, which can be mainly due to the slow evolution of soil parameters in semiarid Mediterranean soils (Bienes et al., 2016). Nevertheless, changes in the 0 to 5 cm depth have been enough for improving hydrologic characteristics like infiltration and runoff (García-Díaz et al., 2017). Our results showed that the GC promotes an increase in soil quality and, as a result, in the soil functioning (Salomé et al., 2014). Permanent covers have increased the organic inputs in the soil system, increasing the SOC stock which has improved the aggregate stability and soil structure. The two GC had a different development. The spontaneous vegetation GC produced higher carbon input through the roots than CB (González-Sánchez et al., 2012), and this produced larger increases of SOC and, as a consequence, bigger improvements in aggregate stability and infiltration rates.

A seeded GC requires an investment in seeds and the use of machinery for soil conditioning. The spontaneous GC does not require this kind of outlays as it can be established directly by stop tilling operations. Moreover, along the trial we noticed herbivory problems with rabbits that showed much higher predilection for the *Brachypodium distachyon* GC. There are researches who have addressed water competition between the plants of GC and the vines in rainfed Mediterranean vineyards producing grape production decreases (Ruiz-Colmenero et al., 2011; Guerra and

Steenwerth, 2012; Muscas et al., 2017). Nevertheless, these studies tend to ignore the value of increasing SOC and erosion reductions on degraded soils.

5. CONCLUSIONS

Alternative soil management strategies such as the use of GC on degraded soils of Mediterranean vineyards improve soil quality in comparison with conventional tillage. Compared to tillage, GC increase soil organic carbon as a consequence of higher carbon inputs. This increase in organic carbon stocks take place in both stable and labile fractions, but is particularly higher in the labile fraction which immediately improves soil fertility. Besides, this indicates that a pool of partially humidified organic matter will be further humified, increasing the stable fractions and contributing to atmospheric CO_2 fixation. SOC stocks increase promotes an improvement of aggregate stability and soil structure making soil more resistant to water erosion. The pore volume and pore size distribution remains similar while the pore connectivity increases dramatically.

WSA was probed more efficient than CND to assess the influence of soil management on soil structure. An increase of soil compaction was confirmed for GC soil management. Higher BD and penetration resistance were recorded for GC compared to T. Nevertheless, this does not necessarily mean a decrease in available water holding capacity or infiltration rates. GC creates a new pore system with microporosity and intrapedal porosity changes which are responsible of these differences. The pace of these changes is different between GC. In this study higher microporosity and more WHC have been measured for CB compared to T. This is particularly remarkable because it can be used as an argument to convince farmers to perform GC. Nevertheless, this observation must be monitored for longer periods of time and for different edaphological and environmental circumstances. This is an important topic to address in future research. Vine age, soil texture or soil depth can have a key role on this. More knowledge is necessary to demonstrate that GC can improve soils without impacts in long term grape production, hence to maintain agrosystem sustainability in Mediterranean areas.

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Table 1. Soil Classification (Soil Survey Staff, 2015) and main soil characteristics of the vineyards. The trial had two vineyards in Belmonte de Tajo municipality (Bel-1 and Bel-2). The soil organic carbon (SOC), pH, electrical conductivity (EC), carbonates, active lime, texture class and cation exchange capacity (CEC) correspond to the Ap horizon.

Site	Soil Classification (USDA)	Altitude (m)	Slope (%)	SOC (gr kg^{-1})	pH (1:2.5)	EC (µS cm^{-1})	Carbonates (%)	Active lime (%)	Clay (%)	Silt (%)	Sand (%)	CEC (meq 100g^{-1})
Bel-1	Calcic Haploxeralf	754	7.2	8.1	8.5	162	27.8	8.7	24.0	31.5	44.5	14.9
Bel-2	Typic Haploxeralf	743	7.0	5.2	8.6	144	20.0	4.4	27.5	38.5	34.0	18.8
Campo Real	Calcic Haploxeralf	783	13.4	7.2	8.6	160	16.1	6.2	31.5	18	50.5	12.2
Navalcarnero	Typic Haploxeralf	586	13.5	7.0	7.7	145	0.1	<0.1	24.5	8.5	67.0	14.0

Table 2

Table 2. Results from the analysis of variance for soil organic carbon (SOC), mineral-associated organic carbon (MOC), soil carbon of the particulate organic matter fraction (POC). The treatments were CB (*Brachypodium distachyon*), CS (Spontaneous Vegetation) and T (Conventional Tillage). Sites were vineyards in Belmonte de Tajo (two vineyards), Campo Real and Navalcarnero. Depth were 0-5 and 5-10 cm. Different letters mean differences between treatments for the 0-10 depth at p<0.05. Terms with p value >0.1 were not reported (NR).

	Treat	**Site**	**Depth**	**Treat* Site**	**Treat* Depth**	**Site* Depth**	**Treat* Site*Depth**
SOC	<0.001	<0.001	<0.001	0.001	0.091	0.014	0.051
MOC	<0.001	<0.001	<0.001	0.011	NR	NR	0.040
POC	0.004	<0.001	0.083	NR	0.047	0.003	0.001

Table 3. Mean and standard deviation for the different treatments and results from the analysis of variance for counting number drops aggregate stability test (CND), water stable aggregates test (WSA), roots stock and bulk density for the different treatments. The treatments were CB (Brachypodium distachyon), CS (Spontaneous Vegetation) and T (Conventional Tillage). Sites were vineyards in Belmonte de Tajo, Campo Real and Navalcarnero. Depths were 0-5 and 5-10 cm. Different letters mean differences between treatments for the 0-10 cm depth at $p<0.05$. Terms with p value >0.1 were not reported (NR).

	Treatment			**Effect**						
	CB	**CS**	**T**	**Treat**	**Site**	**Depth**	**Treat* Site**	**Treat* Depth**	**Site* Depth**	**Treat* Site*Depth**
CND	10.41 ± 0.76 a	20.14 ± 2.67 b	9.14 ± 0.85 a	<0.001	<0.001	NR	0.001	NR	NR	NR
WSA (%)	41.63 ± 1.56 b	43.01 ± 1.81 b	37.11 ± 1.46 a	<0.001	<0.001	<0.001	0.023	NR	NR	NR
Roots (Mg ha^{-1})	7.39 ± 8.04 ab	9.55 ± 11.58 b	6.04 ± 6.02 a	<0.001	<0.001	<0.001	0.019	NR	<0.001	0.036
Bulk density (Mg m^{-3})	1.42 ± 0.02 b	1.37 ± 0.03 ab	1.34 ± 0.02 a	0.049	<0.001	-	NR	-	-	-
Intrapedal porosity (%)	36.61 ± 0.76 b	34.61 ± 0.90 a	34.14 ± 0.84 a	0.011	<0.001	-	0.096	-	-	-

Figure 1

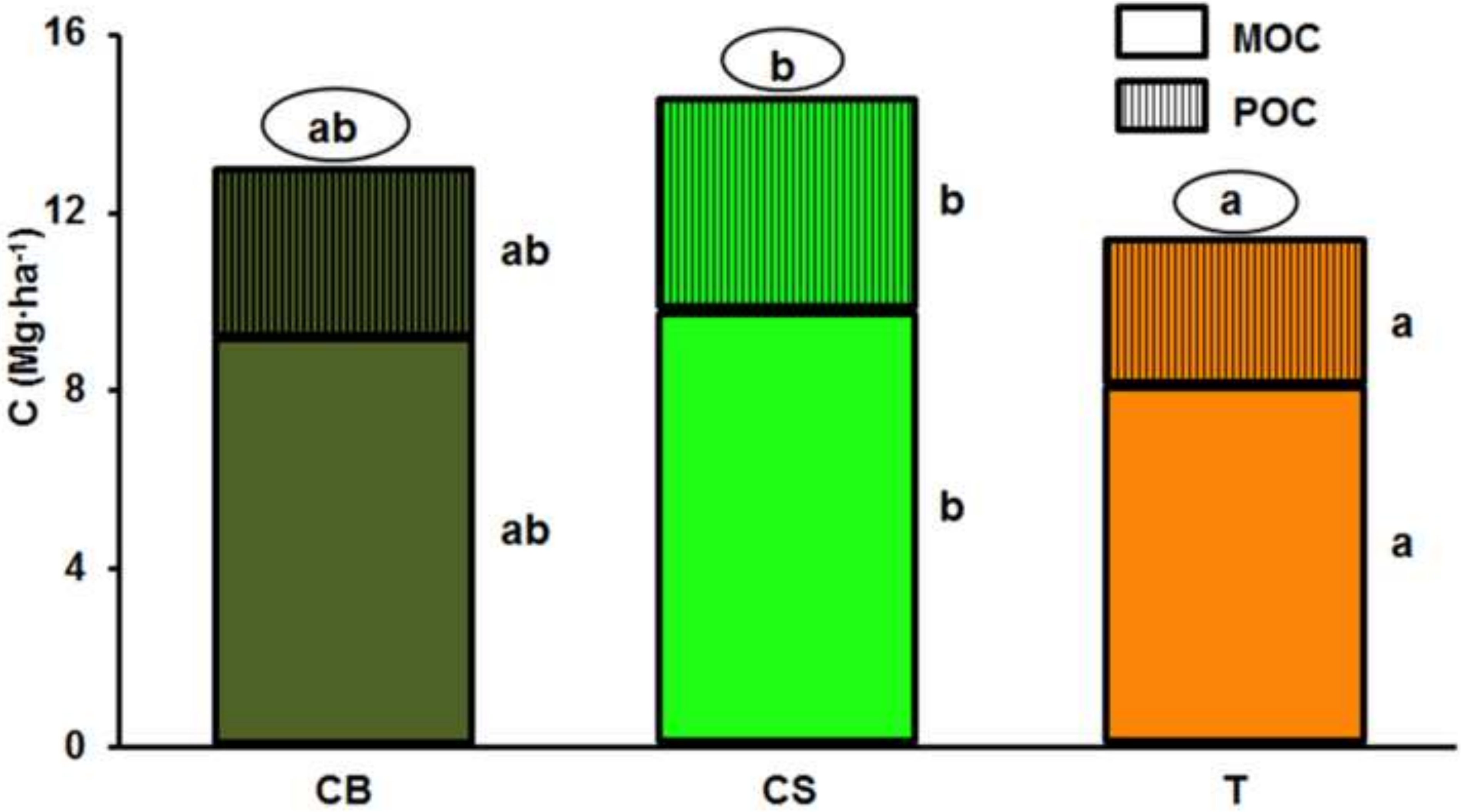


Figure 2

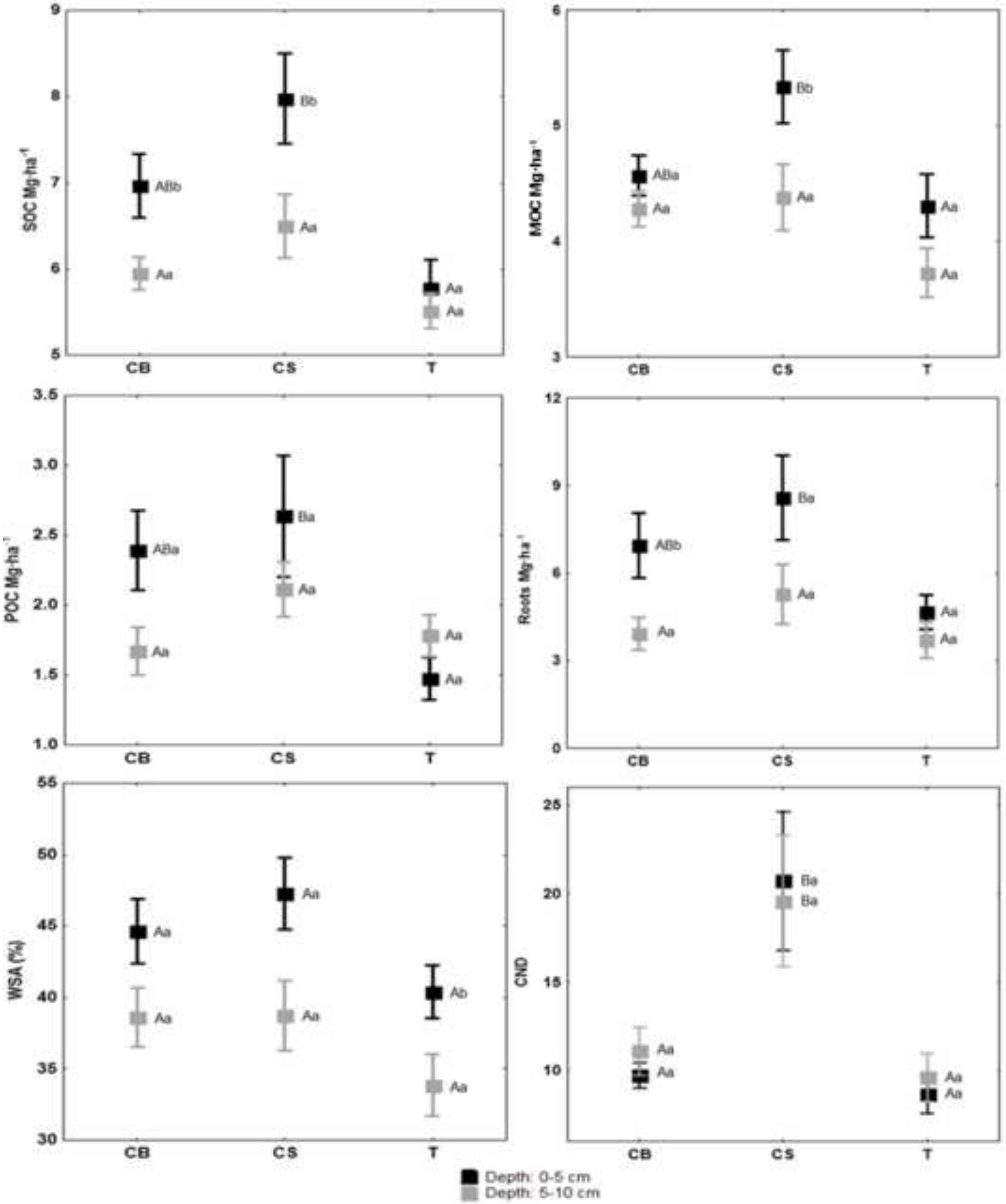

SOC Mg·ha⁻¹
9
8
7
6
5
ABb
Aa
Bb
Aa
Aa
Aa
CB
CS
T
MOC Mg·ha⁻¹
6
5
4
3
ABa
Aa
Bb
Aa
Aa
Aa
CB
CS
T
POC Mg·ha⁻¹
3.5
3.0
2.5
2.0
1.5
1.0
ABa
Aa
Ba
Aa
Aa
Aa
CB
CS
T
Roots Mg·ha⁻¹
12
9
6
3
0
ABb
Aa
Ba
Aa
Aa
Aa
CB
CS
T
WSA (%)
55
50
45
40
35
30
Aa
Aa
Aa
Aa
Ab
Aa
CB
CS
T
CND
25
20
15
10
Aa
Aa
Ba
Ba
Aa
Aa
CB
CS
T
Depth: 0-5 cm
Depth: 5-10 cm

Figure 3

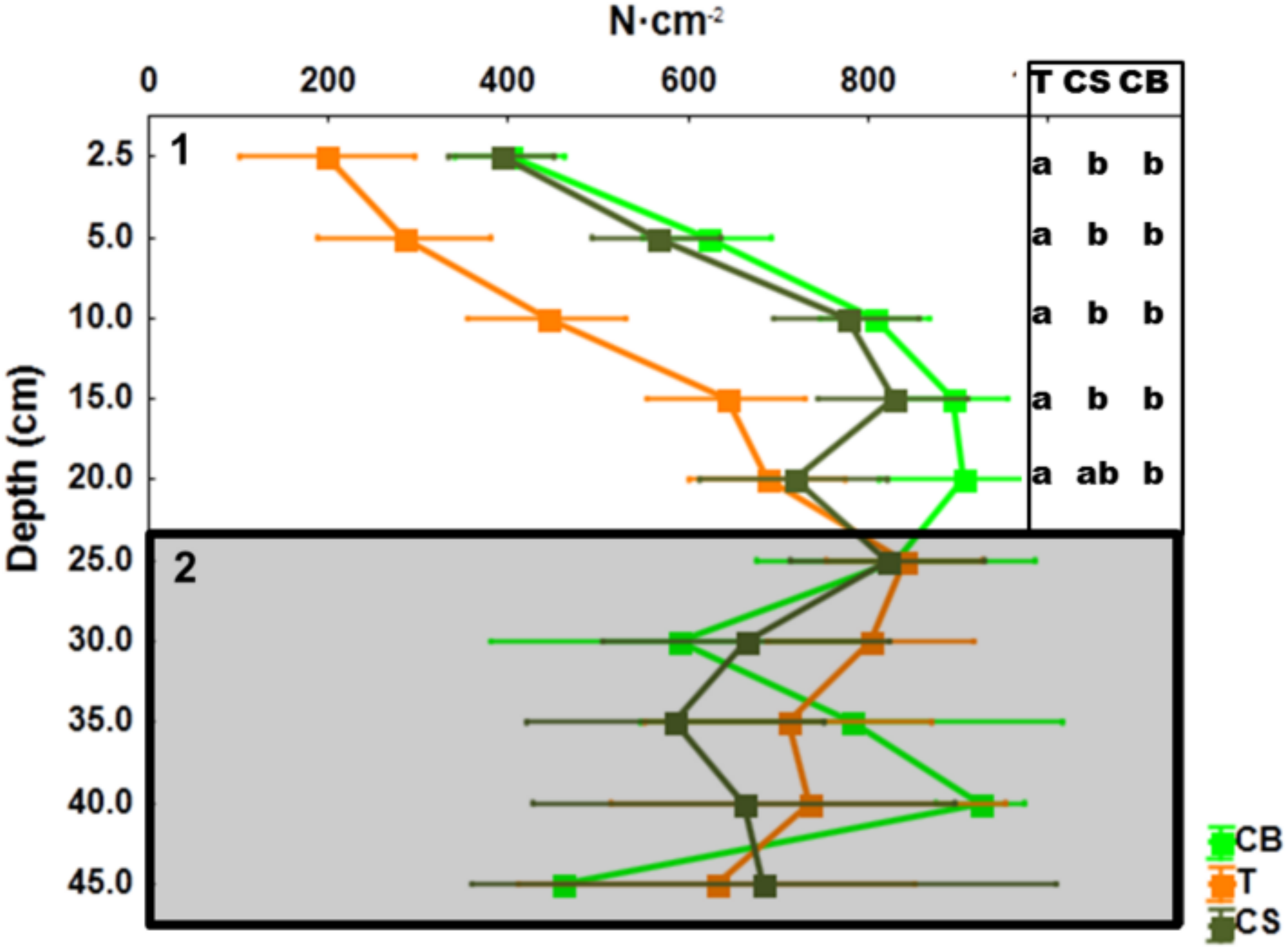

N·cm-2
0
200
400
600
800
Depth (cm)
2.5
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
1
2
T CS CB
a b b
a b b
a b b
a b b
a ab b
CB
T
CS

Figure 4

Click here to download high resolution image

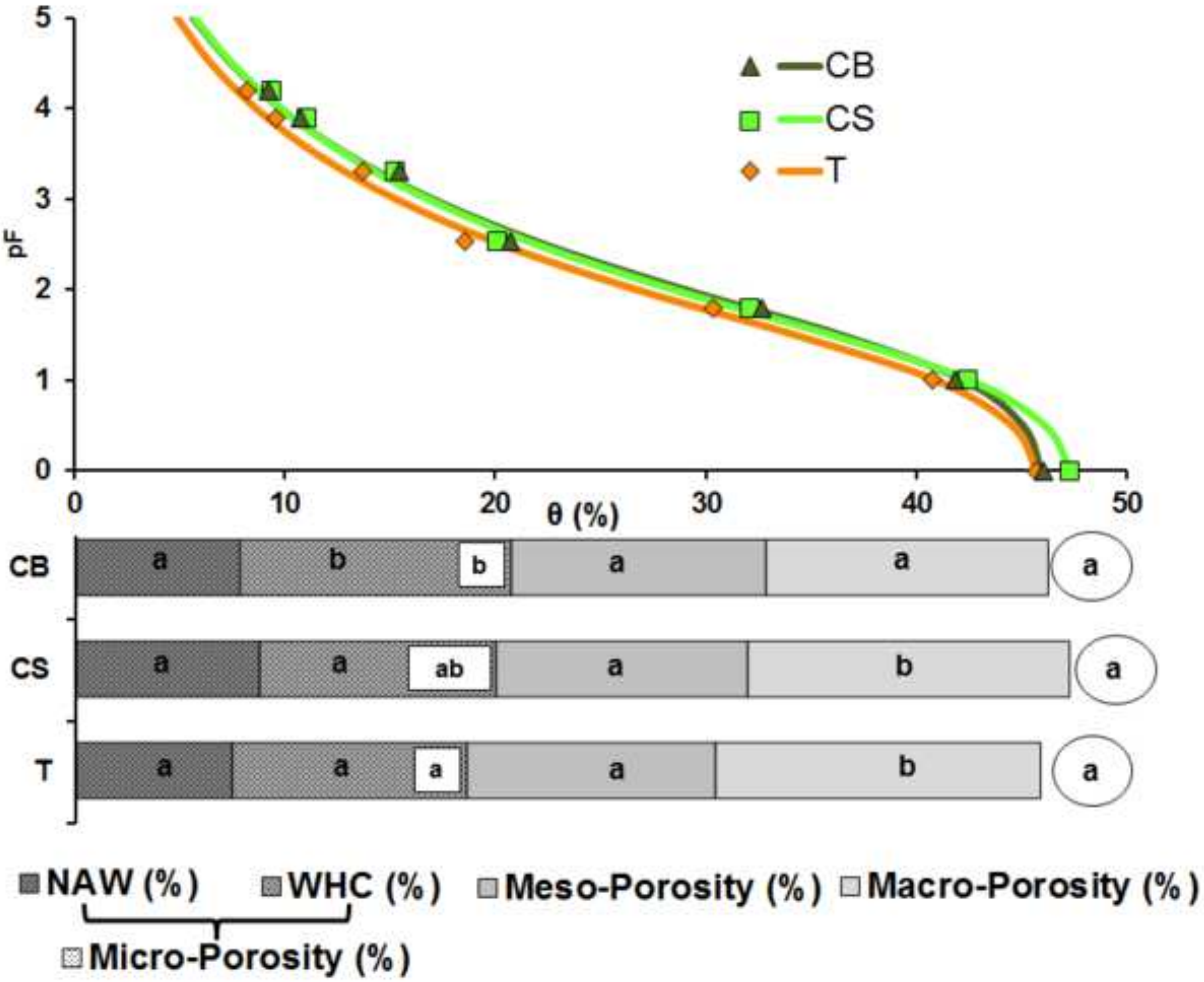
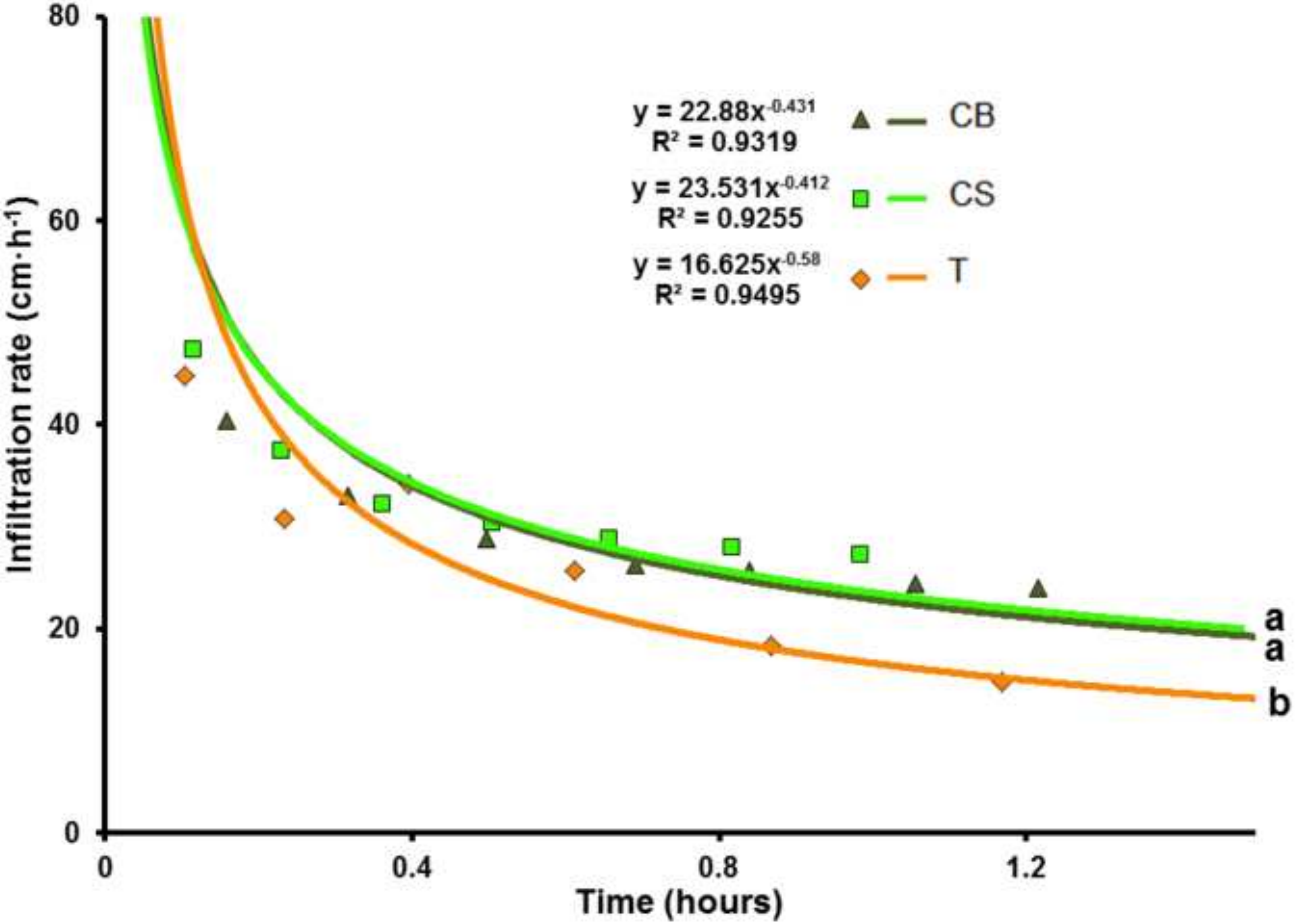


Figure 5



Infiltration rate (cm·h⁻¹)
80
60
40
20
0
0
0.4
0.8
1.2
Time (hours)
y = 22.88x^-0.431
R² = 0.9319
▲ CB
y = 23.531x^-0.412
R² = 0.9255
■ CS
y = 16.625x^-0.58
R² = 0.9495
◆ T
a
a
b

Figure 6

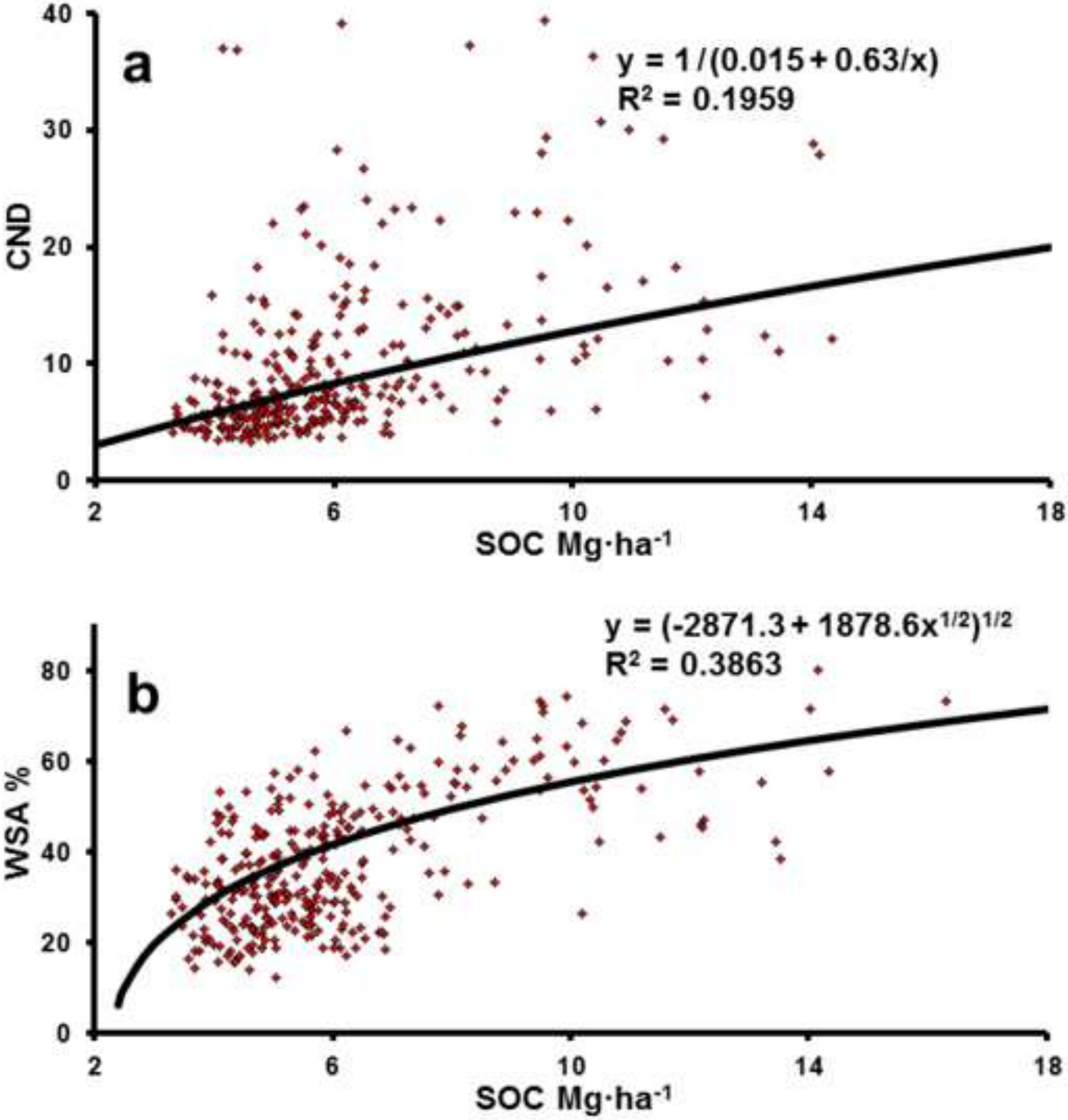
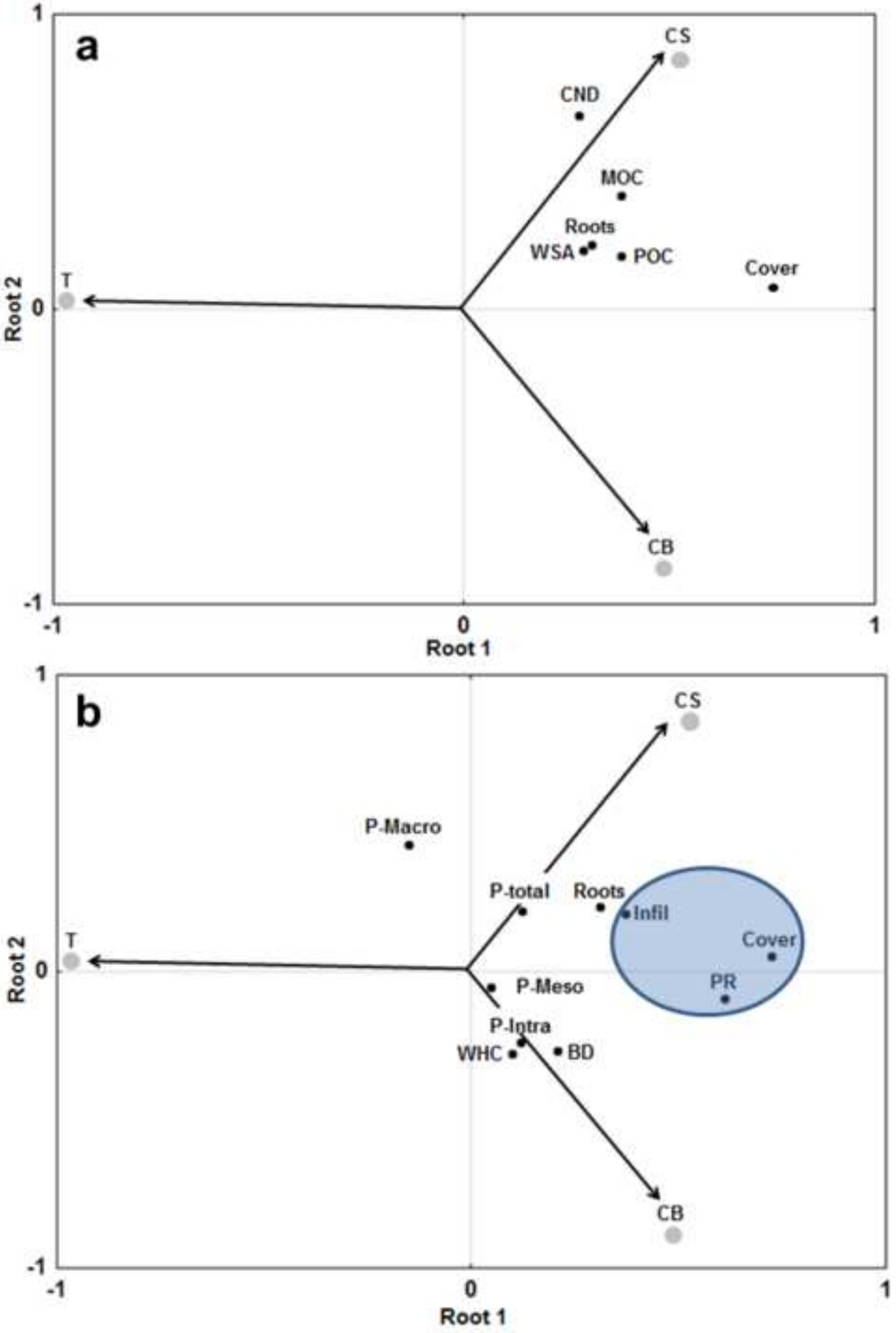

a
y = 1 / (0.015 + 0.63/x)
R² = 0.1959
CND
0
10
20
30
40
2
6
10
14
18
SOC Mg·ha⁻¹
b
y = (-2871.3 + 1878.6x^{1/2})^{1/2}
R² = 0.3863
WSA %
0
20
40
60
80
2
6
10
14
18
SOC Mg·ha⁻¹

Figure 7


a
CS
CND
MOC
Roots
WSA
POC
Cover
T
CB
Root 2
Root 1
1
0
-1
-1
0
1
b
CS
P-Macro
P-total
Roots
Infil
Cover
T
P-Meso
PR
P-Intra
WHC
BD
CB
Root 2
Root 1
1
0
-1
-1
0
1

***Graphical Abstract**